Bacteriocins as an Alternative to Antibiotics

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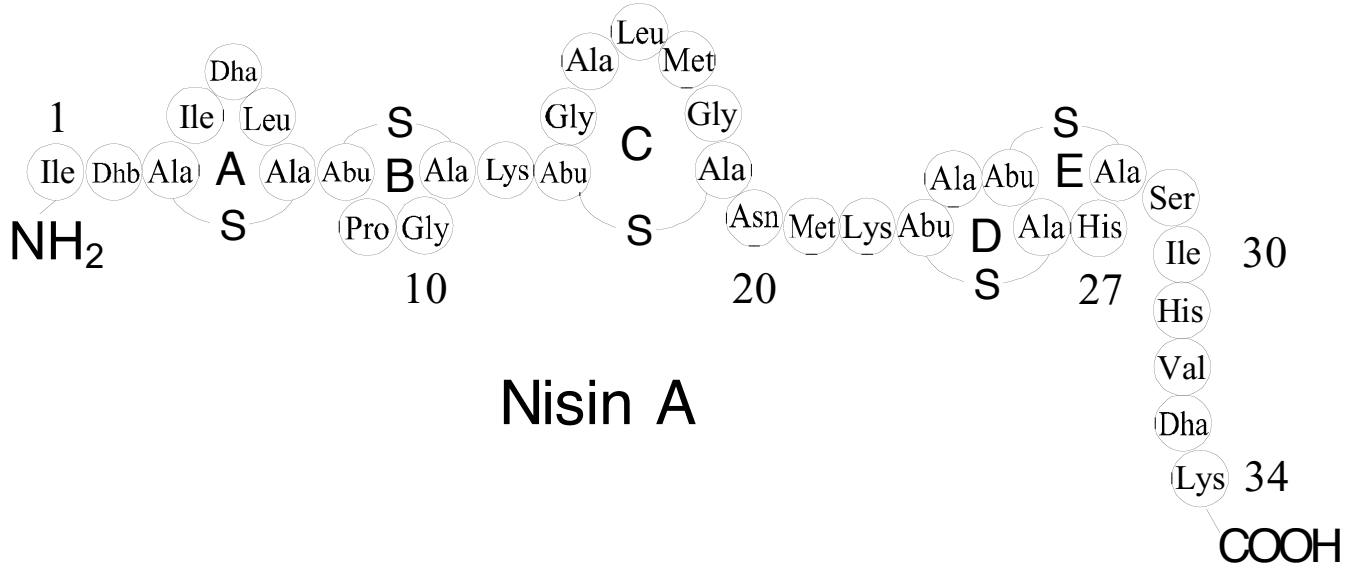
Introduction

Cattle depend on the endproducts of ruminal fermentation for much of their nutrition. Volatile fatty acids are utilized as an energy source from animal metabolism, and microbial protein can supply as much as 90% of the amino nitrogen reaching the small intestine. Ruminal fermentation, however, also produces methane and ammonia, and these end-products are losses of energy and nitrogen.

Research with beef cattle has shown us that Gram-positive antibodies (e.g. ionophores) by altering ruminal fermentation can decrease methane and ammonia and improve the efficiency of feed utilization. However, ionophores have not been approved for use in lactating cattle, and they can be toxic to animals and man. The most commonly used ionophore (monensin) kills horses, and it can cause nausea and headaches in humans that handle it.

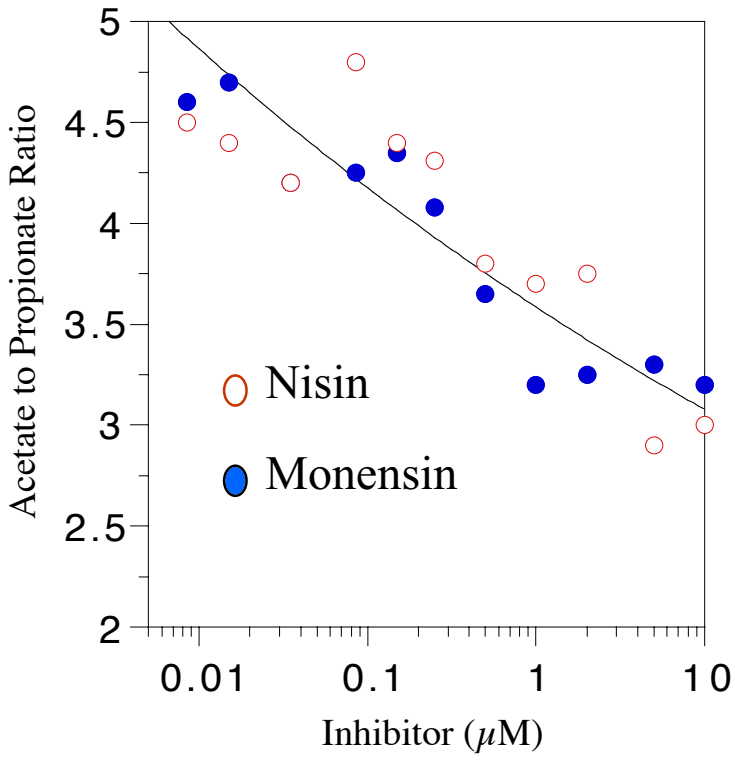
It has long been recognized that many Gram-positive bacteria produce small peptides that inhibit other Gram-positive species. These small peptides (bacteriocins) are not classified as antibiotics and are not thought to be toxic. Indeed, the best studied bacteriocin (nisin) is produced by a cheese starter (*Lactococcus lactis*), and the FDA has given it GRAS status.

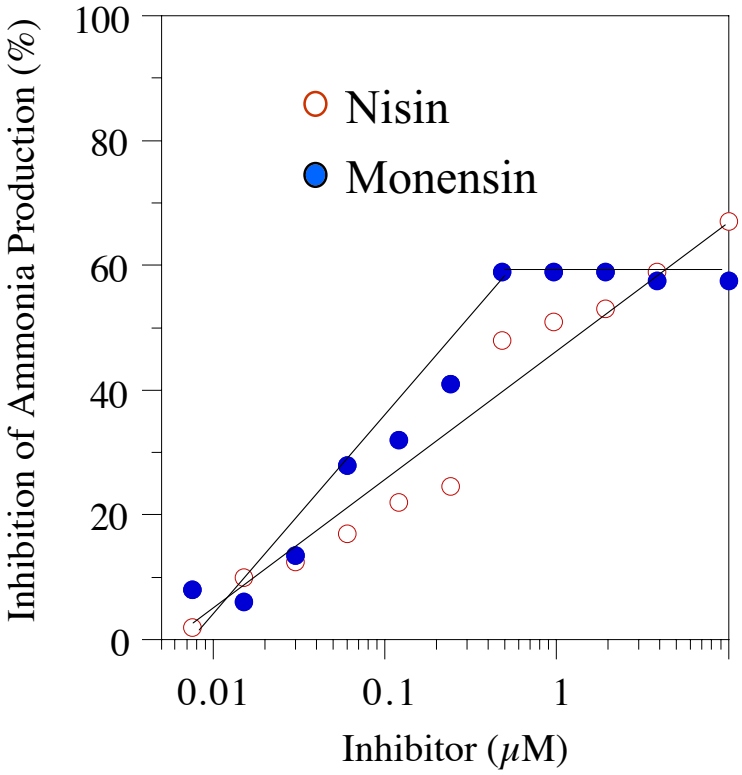
Nisin makes pores in sensitive bacteria



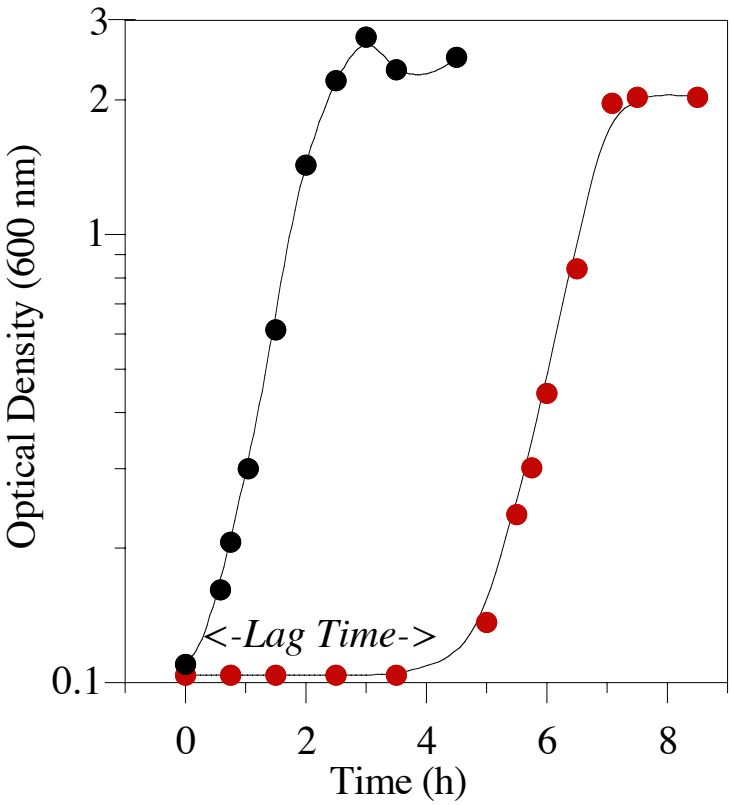
Nisin A

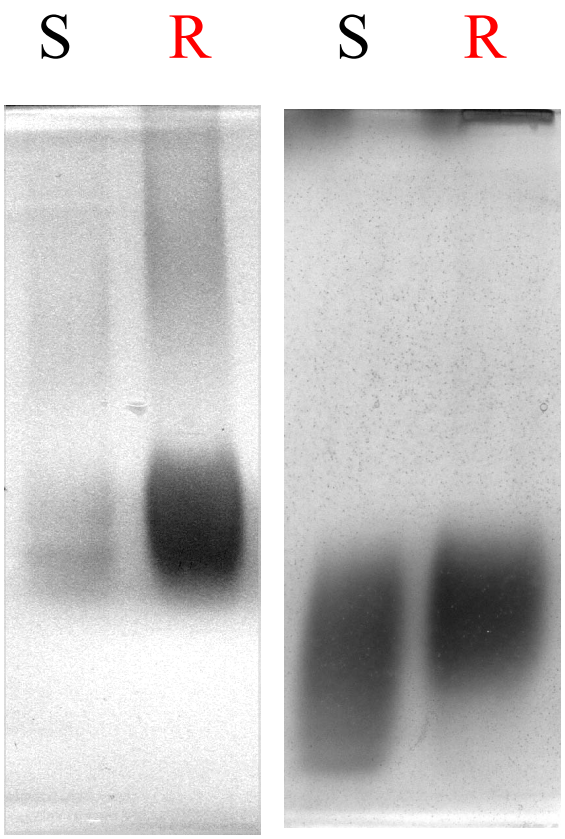
and it has the same activity as monensin in vitro





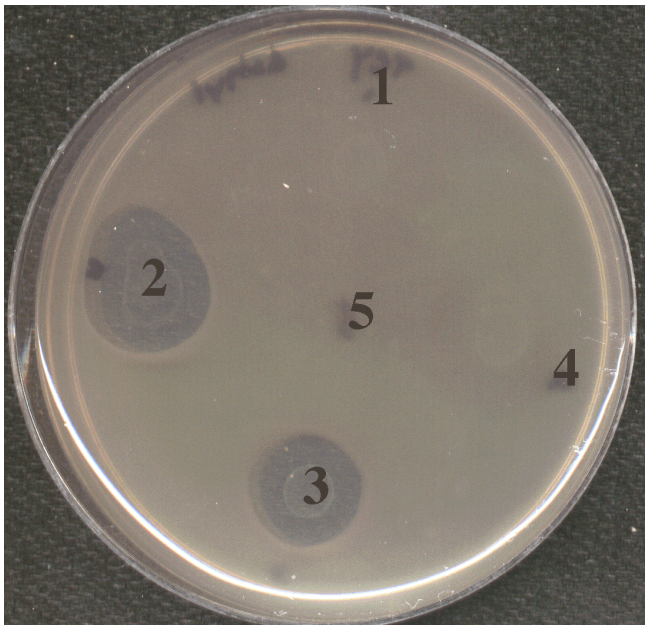
However, some bacteria can change their cell wall and become highly nisin-resistant (R)

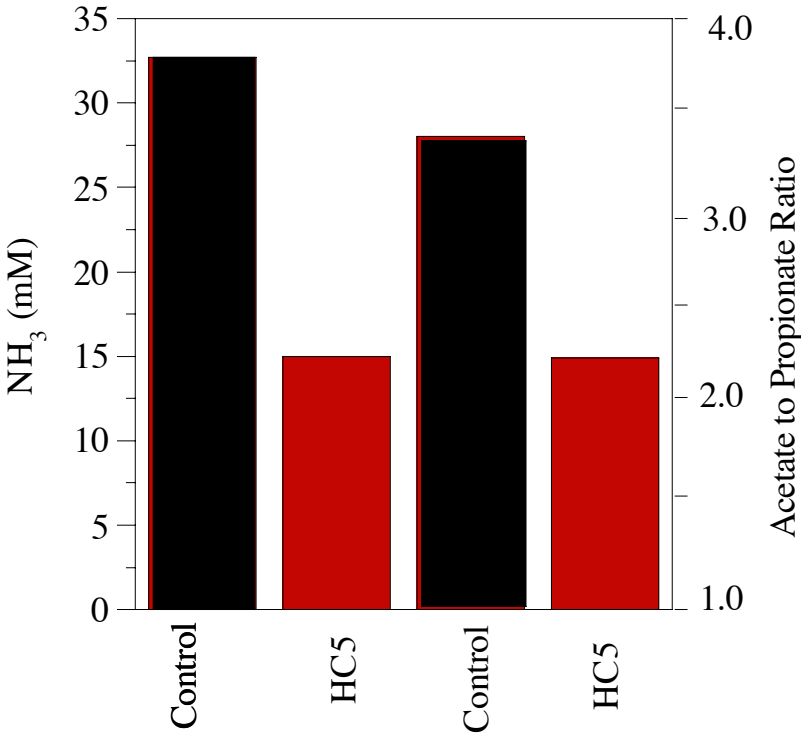




after a short lag time *S. bovis* JB1 grew rapidly even if nisin was present

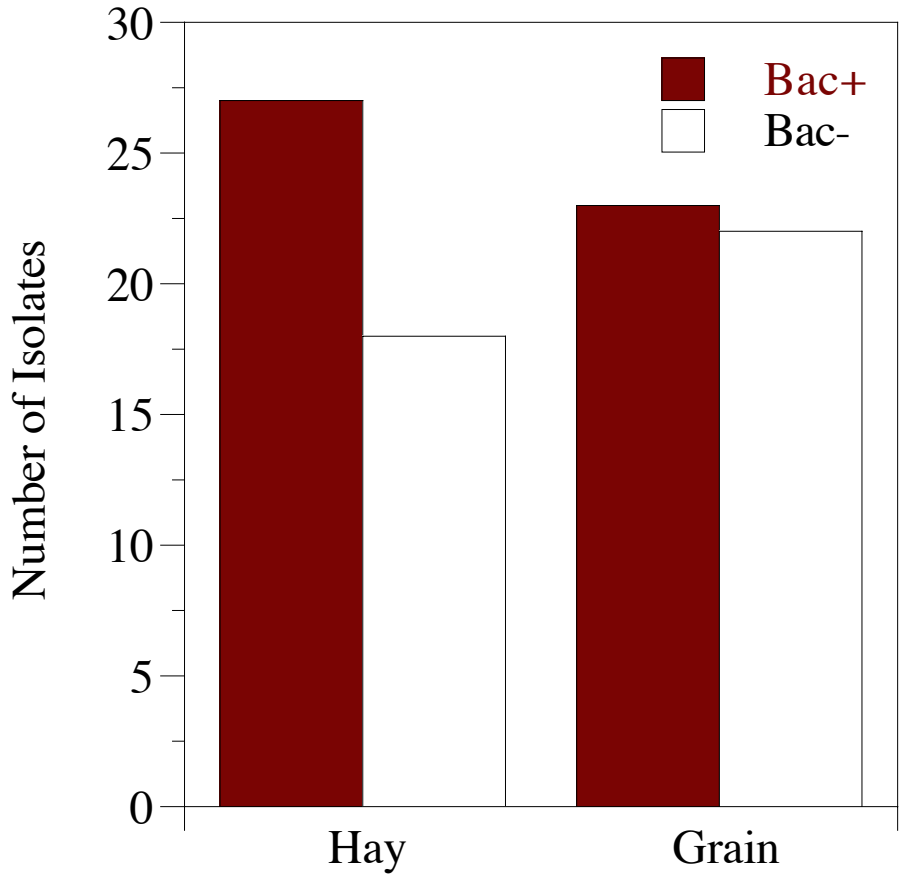
However, some strains of *S. bovis* can produce bacteriocins that are even more active than nisin





The question the arose, can other *S. bovis* strains become resistant to the HC5 bacteriocin?

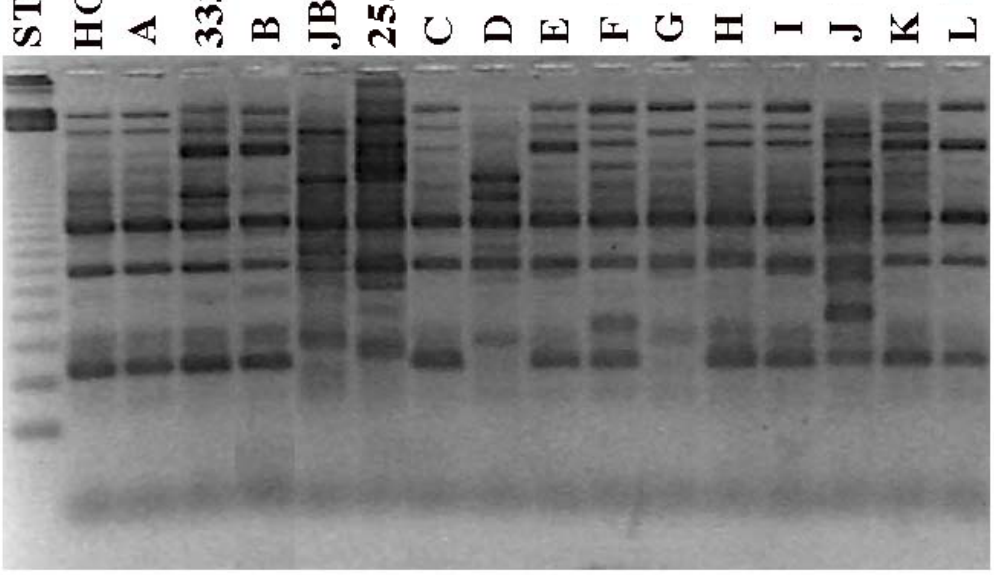
In order to answer this question, we isolated 90 strains of *S. bovis* from the rumen.



Approximately 50% of the strains produced a bacteriocin that could inhibit *S. bovis* JB1.

By using repetitive DNA (BOX) sequences we were ale to classify the strains and determine their homology.

a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JB1
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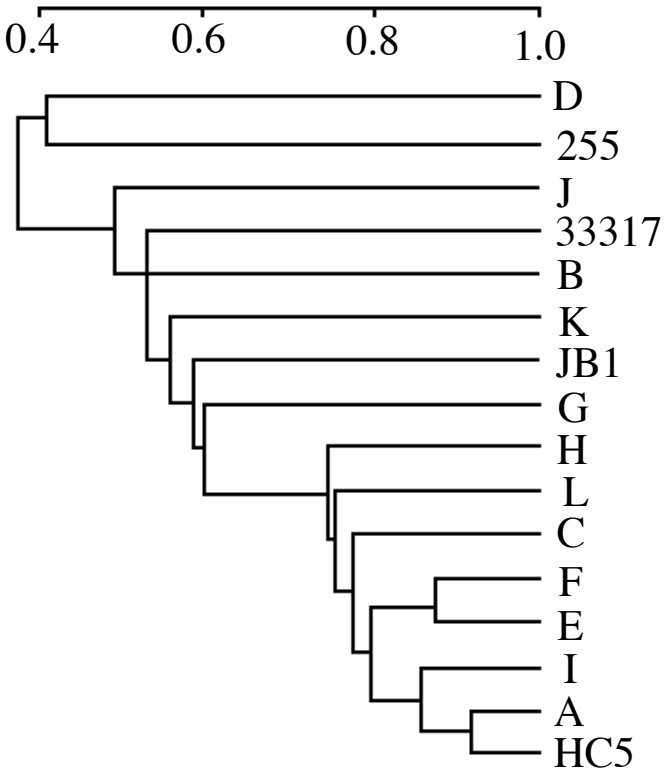
b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A
HC5

When repetitive DNA (BOX) sequences were amplified by PCR, the bacteriocin-producing (bac^+) strains could be organized into 16 phylogenetic groups, but the similarity indexes (UPGAMA) were as low as 40%. Based on these results, bacteriocin production was not a phylogenetically conserved trait.

The isolates were sensitive to the bacteriocin of *S. bovis* HC5, the decrease in viability was approximately 3 logs greater (4.8 ± 2.7), and the HC5-sensitive strains did not adapt.

Sixteen of the bac^+ isolates were highly resistant to *S. bovis* HC5 ($\leq$ 1 log reduction in viability), and BOX PCR indicated that only 5 of them had the same BOX pattern as *S. bovis* HC5. The remaining bac^+, HC5-resistant isolates (n=11) had distinctly different BOX patterns.

The Silo as a Delivery System

S. bovis is not an ideal ruminal bacterium, and it can cause ruminal acidosis if the diet has an abundance of soluble sugar or starch. However, the rapid growth and lactate production of *S. bovis* makes it an ideal silage inoculant.

When Richard Muck compared *S. bovis* strains with commercial strains of lactobacilli in "mini silos," *S. bovis* caused more rapid declines in pH and increases in lactic acid than commonly used lactobacilli.

Amino acid degradation is an undesirable feature of silage fermentation that buffers the decrease in pH, wastes amino acids, and causes undesirable odors (butyric acid and amines).

Preliminary experiments indicate that *S. bovis* HC5 can kill ruminal bacteria with very high rates of amino acid discrimination, and it is conceivable that it could inhibit the silage clostridia that ferment amino acids.

Hypotheses

Given the information that is currently available we have formulated the following hypotheses:

(1) bacteriocin producing *S. bovis* strains could be used as silage inoculants;

(2) the bacteriocins could improve silage quality by increasing lactic acid and decreasing amino acid degradation;

(3) bacteriocins remaining in the treated silage could have a beneficial effect on ruminal fermentation.